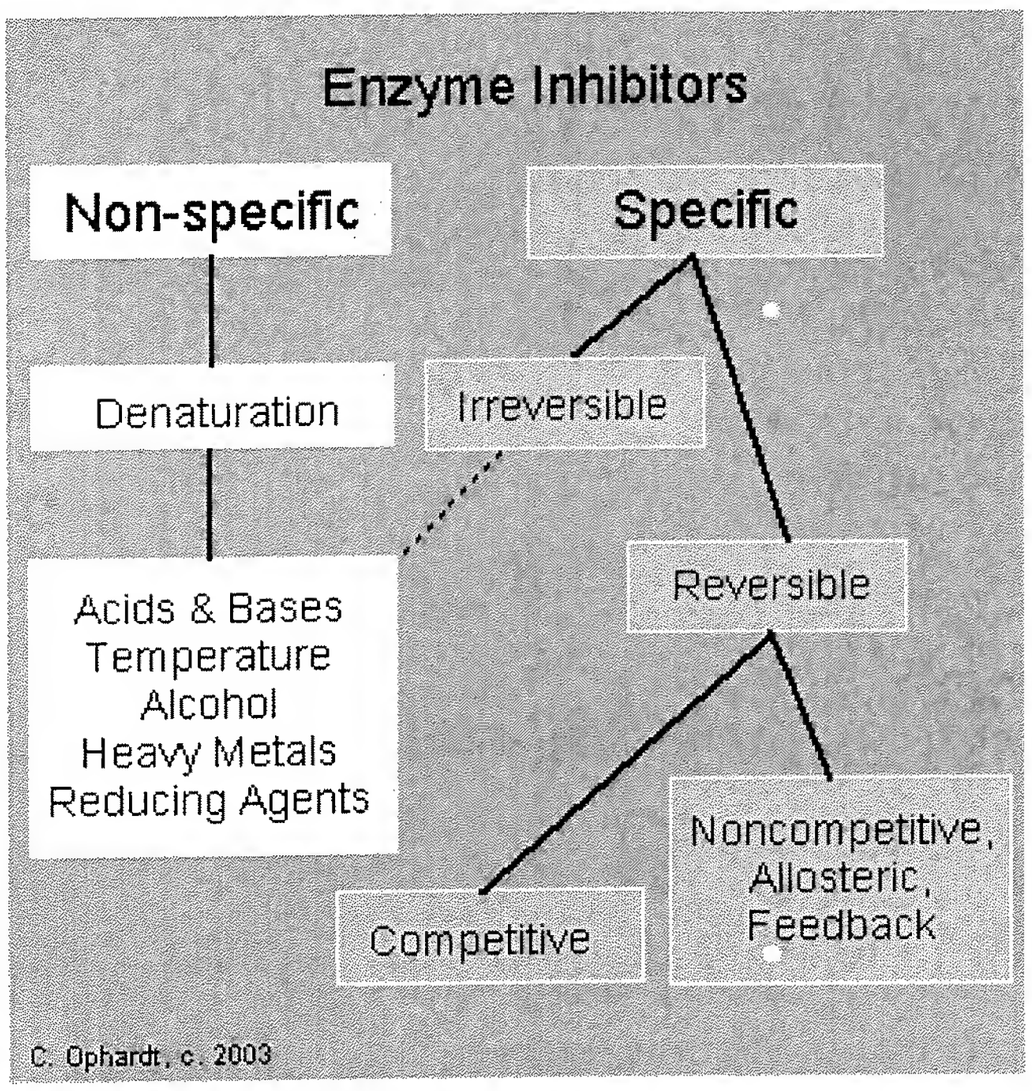
EXHIBIT A



Enzyme Inhibitors

Introduction:

Enzyme inhibitors are molecules that interact in some way with the enzyme to prevent it from working in the normal manner. There are a variety of types of inhibitors including: nonspecific, irreversible, reversible - competitive and noncompetitive. Poisons and drugs are examples of enzyme inhibitors.

Nonspecific Inhibitors:

A nonspecific inhibition effects all enzymes in the same way. Non-specific methods of inhibition include any physical or chemical changes which ultimately **denatures** the protein portion of the enzyme and are therefore irreversible.

Temperature: Usually, the reaction rate increases with temperature, but with enzyme reactions, a point is reached when the reaction rate decreases with increasing temperature. At high temperatures the protein part of the enzyme begins to denature, thus inhibiting the reaction.

Quiz: What happens to the active site and the molecular geometry of the enzyme as it is denatured?	Answer

Acids and Bases: Enzyme activity is also controlled by pH. As the pH is decreased or increased, the nature of the various acid and amine groups on side chains is altered with resulting changes in the overall shape structure of the enzyme.

Quiz: Excess of either acid or base causes denaturing of protein. What type of bonds are disrupted by this action?	Answer
Name some other denaturing methods to inhibit or destroy enzyme activity.	Answer
If an enzyme has been denatured, is it likely that its enzyme activity can be	

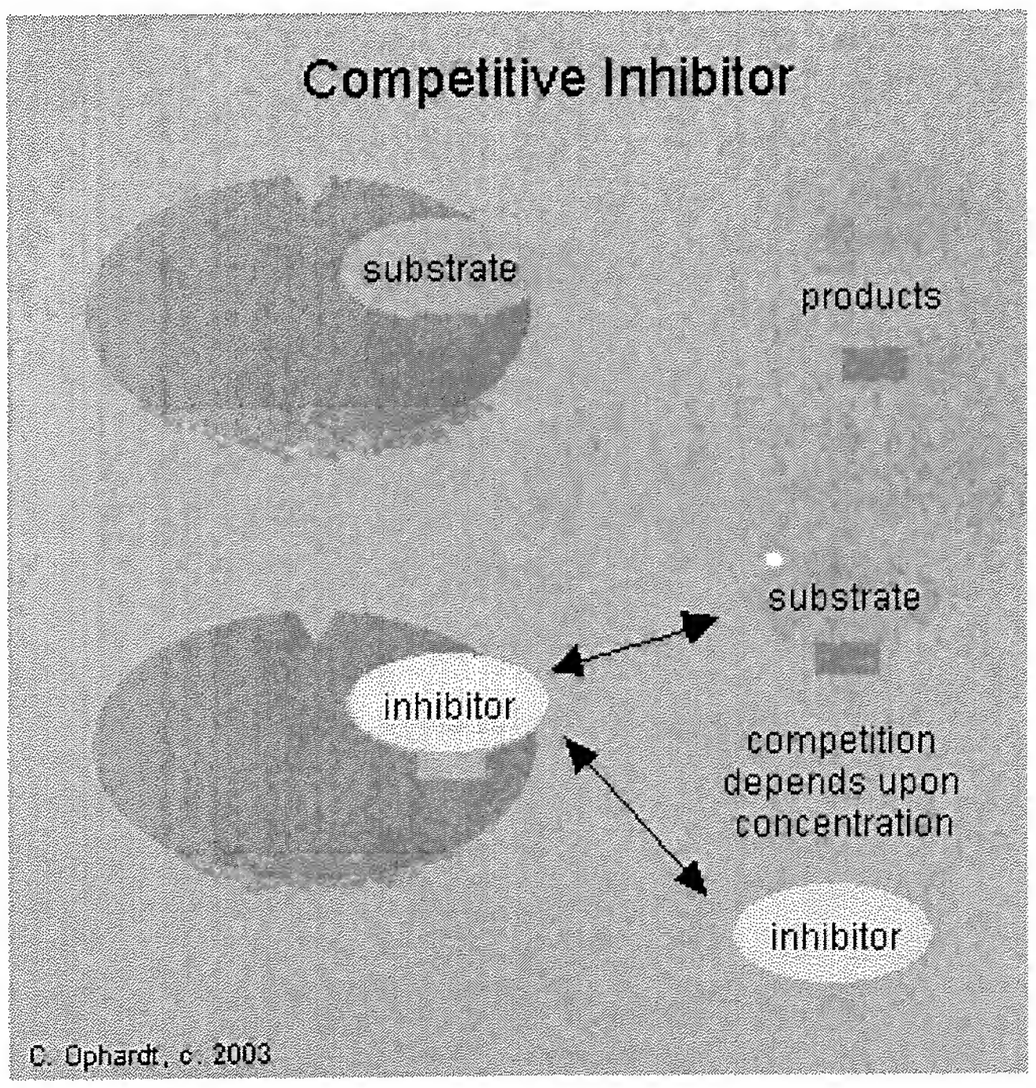
restored? Is the inhibition reversible or irreversible? Explain.	Answer

Specific Inhibitors:

Specific Inhibitors exert their effects upon a single enzyme. Most poisons work by specific inhibition of enzymes. Many drugs also work by inhibiting enzymes in bacteria, viruses, or cancerous cells and will be discussed later.

Competitive Inhibitors:

A competitive inhibitor is any compound which closely resembles the chemical structure and molecular geometry of the substrate. The inhibitor competes for the same active site as the substrate molecule. The inhibitor may interact with the enzyme at the active site, but no reaction takes place. The inhibitor is "stuck" on the enzyme and prevents any substrate molecules from reacting with the enzyme. However, a competitive inhibition is usually reversible if sufficient substrate molecules are available to ultimately displace the inhibitor. Therefore, the amount of enzyme inhibition depends upon the inhibitor concentration, substrate concentration, and the relative affinities of the inhibitor and substrate for the active site.



Quiz: If the concentration of inhibitor is less than that of the substrate and the substrate has a higher affinity for the active site, is the enzyme inhibited a lot, or a little?	Answer
If the concentration of inhibitor is more than that of the substrate is the enzyme inhibited a lot, or a little?	Answer

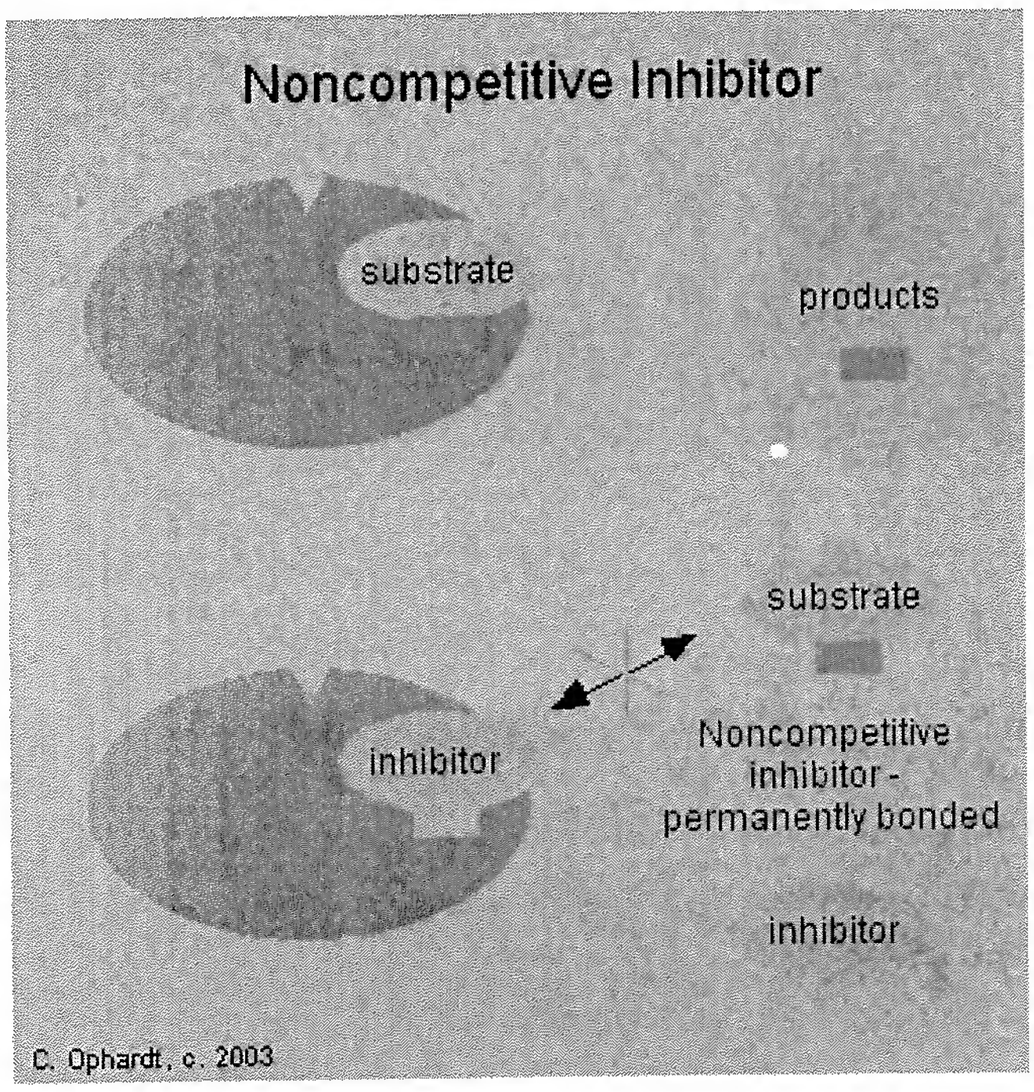
Example: Ethanol is metabolized in the body by oxidation to acetaldehyde, which is in turn further oxidized to acetic acid by aldehyde oxidase enzymes. Normally, the second reaction is rapid so that acetaldehyde does not accumulate in the body.

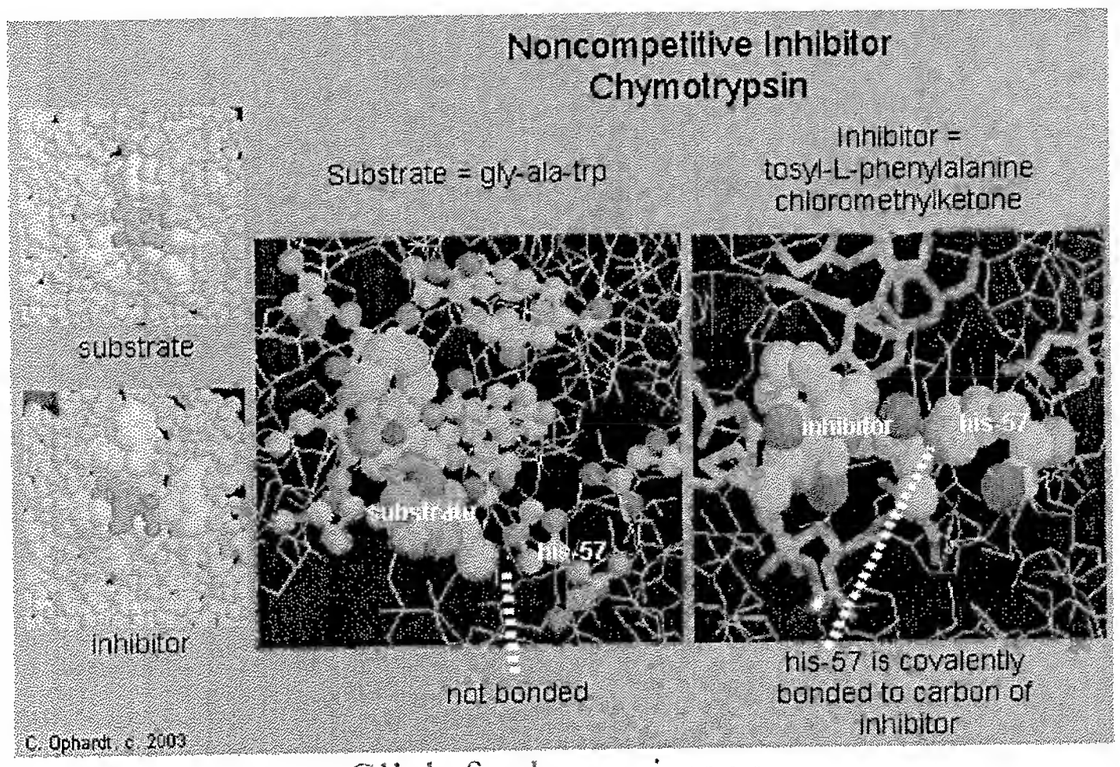
A drug, **disulfiram (Antabuse) inhibits** the aldehyde oxidase which causes the accumulation of acetaldehyde with subsequent unpleasant side-effects of nausea and vomiting. This drug is sometimes used to help people overcome the drinking habit.

Methanol poisoning occurs because methanol is oxidized

to formaldehyde and formic acid which attack the optic nerve causing blindness. Ethanol is given as an antidote for methanol poisoning because ethanol competitively inhibits the oxidation of methanol. Ethanol is oxidized in preference to methanol and consequently, the oxidation of methanol is slowed down so that the toxic by-products do not have a chance to accumulate.

QUES. **Ethylene glycol**, if ingested, can be poisonous. Ethylene glycol is oxidized by the same enzymes used in the previous examples by ethanol and methanol. Describe how ethanol can be used as an antidote.





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Noncompetitive Inhibitors:

A noncompetitive inhibitor is a substance that forms strong covalent bonds with an enzyme and consequently may not be displaced by the addition of excess substrate. Therefore, noncompetitive inhibition is irreversible. A noncompetitive inhibitor may be bonded at, near, or remote from the active site. In any case, the basic structure of the enzyme is modified to the degree that it ceases to work. See the graphic on the left.

If the inhibition is at a place remote from the active site, this is called allosteric inhibition. Allosteric means "other site" or "other structure". The interaction of an inhibitor at an allosteric site changes the structure of the enzyme so that the active site is also changed.

Since many enzymes contain sulfhydral (-SH), alcohol, or acid groups as part of their active sites, any chemical which can react with them acts as a noncompetitive inhibitor. Heavy metals such as Ag^{+}, Hg^{2+}, Pb^{2+} have strong affinities for -SH groups.

Nerve gases such as diisopropylfluorophosphate (DFP) inhibit the active site of acetylcholine esterase by reacting with the hydroxyl group of serine to make an ester.

Oxalic and citric acid inhibit blood clotting by forming complexes with calcium ions necessary for the enzyme metal ion activator.

QUES. Explain a method that could be used to experimentally test whether an inhibitor was competitive or noncompetitive. Hint: Consider concentration effects and reversible vs. irreversible effects.

Example: Chymotrpsin is an enzyme which hydrolyzes peptides at the carbonyl side of tyr or phe or trp (i.e. those that have an aromatic side chain. In the graphic on the left, the substrate and the inhibitor are shown in the active site pocket. In the case of the inhibitor the reaction starts in the same way as with the substrate, but the end result is that the inhibitor is covalently bonded to the histidine-57

	in the active site and is not reversible. Chymotrypsin with substrate - Chime in new window Chymotrypsin with inhibitor - Chime in new window